bio-protocol Preprint

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Subcellular fractionation

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An abbreviated version of this protocol was published in eLIFE in Dec 2018

The transcription factors TFE3 and TFEB amplify p53 dependent transcriptional programs in response to DNA damage

DOI: 10.7554/eLife.40856

Detailed protocol

Cells were lysed in NP-40 lysis buffer (l0 mM Tris, pH 7.9, 140 mM KCl, 5 mM $MgCl_2$and 0.5 % NP-40) supplemented with protease and phosphatase inhibitors at a concentration of 3-5x10^7 cells/ml. Cell were gently mixed and kept on ice for 15 min. The lysates were then centrifugated at 1000 x g for 5 min. The supernatant represents the cytosolic plus the membrane fraction. The pellets (nuclear fraction) were washed twice in NP-40 lysis buffer and then sonicated in Laemmli sample on ice using a Misonix sonicator Processor XL2020 equipped with a horn/probe (fine tip). The sonication processing time was 1 min with pulses of 10 sec every 5 sec intervals and power setting at position 2.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Puertollano, R. (2020). Subcellular fractionation. Bio-protocol Preprint. bio-protocol.org/prep223.
2. Brady, O. A., Jeong, E., Martina, J. A., Pirooznia, M., Tunc, I. and Puertollano, R.(2018). The transcription factors TFE3 and TFEB amplify p53 dependent transcriptional programs in response to DNA damage. eLIFE. DOI: 10.7554/eLife.40856